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Effect of obesity on the expression of the organic anion transporting polypeptide Oatp1a1 in mouse liver

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1 Abstract

Obesity poses a serious human health risk. Obese patients usually take prescription drugs that require hepatic and renal metabolism and transport, and these patients sometimes display different pharmacokinetics of these drugs. As demonstrated in animal studies, metabolic disturbances such as diabetes or obesity can significantly affect renal and hepatic gene expression including xenobiotic transporters. Moreover, reduced renal mass seems to enhance the changes in gene expression. In this study we analyzed the impact of diet-induced obesity and uninephrectomy on renal and hepatic gene expression. mRNA level of OATPs (organic anion transporting polypeptides) and FXR (farnesoid X receptor) target genes were measured in kidneys and livers of female C57/BJ mice. To assess the effect of diet-induced obesity in a state of reduced renal mass, mice underwent uninephrectomy followed by either a normal chow or high-fat diet and were compared with sham-operated control mice. Gene expression analysis was performed using real-time PCR (polymerase chain reaction). We observed that the hepatic and renal mRNA expression of OATPs is affected mainly by the high-fat diet. Uninephrectomy had no significant impact on renal and hepatic gene expression in this study. Immunohistochemical staining of Oatp1a1 indicates that the decreased mRNA expression was also consistent at the protein level. Additionally, in silico analysis indicates that not only FXR, but also other transcription factors such as PPARα (peroxisome proliferator-activated receptor alpha) is directly or indirectly involved in the regulation of OATPs. In summary, the expression of a number of xenobiotic transporters and FXR regulated genes was significantly altered in livers and kidneys of diet-induced obese mice. Because transporter genes are frequently regulated by similar mechanisms in mouse and human, the data from this study suggest that transporter expression in human liver and kidney may be affected by obesity, leading to altered pharmacokinetics and potentially drug-drug interactions.

2 Introduction

The growing epidemic of obesity is certainly one of the biggest challenges in health care this century. The prevalence of obesity has almost doubled between 1980 and 2008 and is still increasing. According to the WHO (World Health Organization), obesity is among the leading risk factors for mortality worldwide [1]. It is well known that obesity is associated with several comorbidities such as diabetes, hypertension and dyslipidemia. Obesity not only affects the cardiovascular system, but also organs such as the liver and kidney. Non-alcoholic fatty liver disease (NAFLD) is known to be frequently associated with obesity, type 2 diabetes and dyslipidemia [2, 3]. Moreover, obesity is considered an independent risk factor for the development of chronic kidney disease (CKD) and progression to end-stage renal disease [4, 5]. Obesity is a major risk factor for CKD in patients with reduced renal mass [6]. Moreover, metabolic disturbances such as diabetes or obesity can significantly affect renal and hepatic gene expression [7, 8]. Global gene expression analysis in the mouse kidney revealed a substantial number of differentially expressed genes in obese mice after a 20-week high-fat diet. In addition to genes involved in lipid metabolism, cytoskeleton remodeling and fibrosis, genes from the organic anion transporting polypeptide (OATP) transporter family showed altered expression in diet-induced obesity in mice. Furthermore, the effect of a high-fat diet on gene expression was accentuated in obese mice with a reduced renal mass (uninephrectomy). According to pathway analysis, the farnesoid X receptor (FXR) signaling pathway was shown to be one of the top pathways that was activated in the remnant kidney of obese mice [8].

FXR (gene name NR1H4) is a member of the nuclear receptor family and functions as a ligand-activated transcription factor. FXR is mainly expressed in the liver and the intestine, and plays a critical role in bile acid (BA) homeostasis by regulating BA synthesis, BA uptake into hepatocytes, biliary BA secretion, intestinal BA reabsorption and secretion. FXR is activated by free or conjugated bile acids, among which chenodeoxycholic acid (CDCA) is the most potent ligand. The activated FXR can serve as a transcriptional activator or repressor of gene expression. FXR activation increases BA conjugation, BA efflux from the hepatocytes into bile and inhibits hepatic BA synthesis as well as intestinal BA uptake [9, 10]. BAs exert an important role in regulation of cholesterol and triglyceride metabolism. Bile acid sequestrants in the enterohepatic circulation increase both bile acid and VLDL triglyceride synthesis. Conversely, CDCA-mediated increases in the BA pool

size leads to inhibition of BA synthesis and reduced serum triglycerides in hyperlipidemic patients [11, 12]. These effects of BA to modulate triglyceride metabolism are likely mediated via several distinct mechanisms, predominantly activation of FXR. FXR alters the transcription of several genes involved in fatty acid and triglyceride synthesis and lipoprotein metabolism [11]. Thus, FXR may play a critical role under obese conditions.

Liver and kidney play critical roles in drug metabolism and elimination. While liver is the principal site of detoxification and degradation, the kidney is mainly involved in excretion of drugs. Hepatic drug elimination consists of (1) uptake from the portal blood, (2) metabolism and (3) biliary excretion. Drug clearance in the kidney is determined by (1) glomerular filtration, (2) tubular secretion and (3) reabsorption [13]. The cellular uptake and excretion of endo- and xenobiotics are mediated through a variety of membrane transporters. Transporters expressed in mammalian key organs like liver, kidney and the small intestine are important determinants of drug absorption, distribution and elimination [13, 14]. According to their function, transporter proteins can be classified into uptake and efflux transporters. Whereas transporters mediating the uptake into the cell belong to the SLC (solute carriers) superfamilies, most efflux proteins are members of the ABC (ATP-binding cassette) transporter superfamily [14, 15]. Within the SLC transporters, the organic anion transporting polypeptides (OATPs, gene name SLCO), organic cation/anion transporters (OCTs and OATs, gene name SLC22A), and the oligopeptide transporters (PEPTs, gene name SLC15A) are considered the most important subfamilies with pharmacological relevance [16]. OATPs are a group of membrane transporter proteins with a wide spectrum of substrates. They mediate the sodium-independent transport of amphipathic compounds such as bile salts, steroid conjugates, thyroid hormones, anionic oligopeptides, numerous drugs and other xenobiotic substances. Although some members in rodents and humans are predominantly, if not exclusively expressed in liver, most OATP/Oatp family members are expressed in multiple tissues including brain, kidney and intestine [17, 18]. Due to their wide substrate spectrum and their expression in several tissues, Oatps are important for the absorption, distribution, and excretion of drugs. Therefore, altered expression of these uptake transporters may affect drug transport kinetics, which in turn affects drug response in different patients.

Among other nuclear receptors, FXR modulates a wide range of target genes including endo- and xenobiotic transporters from the OATP families [19, 20]. Since these transporters play important roles in drug disposition and elimination, an alteration in their expression can considerably change drug pharmacokinetics leading to the loss of drug efficacy or unexpected toxicity [21]. Given these observations and the rising number of obese people and obese living kidney donors in the world population [22], proper investigation of these transporters and their regulation under obese and uninephrectomized conditions using a rodent model will provide valuable information to aid in the prediction of drug efficacy, pharmacokinetics, and toxicity in morbidly obese patients and living kidney donors.

In the present study we analyzed the impact of diet-induced obesity and uninephrectomy on the renal and hepatic expression of OATPs as well as other FXR target genes. High-fat-diet-fed mice were used as an obesity model. We compared gene expression between sham operated mice fed with chow diet (sham-chow, control), sham operated mice fed with high-fat diet (sham-HFD), uninephrectomized mice with chow diet (UNX-chow) and UNX mice with high-fat diet (UNX-HFD). We observed that the hepatic and renal gene expression of OATPs is mainly affected by the high-fat diet. Uninephrectomy had no significant impact on renal and hepatic gene expression in this study. Immunohistochemical staining of Oatp1a1 indicated that changes in mRNA expression were also detectable at the protein level. Additionally, in silico analysis indicates that not only FXR, but also other transcription factors such as PPARα is directly or indirectly involved in the regulation of OATPs.

3 Material and Methods

3.1 Animals

Female C57/BJ mice were randomly assigned into 4 groups of 6 mice: sham procedure with chow diet (sham-chow/control), sham with high fat diet (sham-HFD), uninephrectomy (UNX) with chow diet (UNX-chow) and UNX with high fat diet (UNX-HFD). Uninephrectomy and sham procedure were performed at the age of 6 weeks. Thereafter, the mice received either a normal chow diet (Provimi Kliba AG, Kaiseraugst, Switzerland) or a high-fat diet (Provimi Kliba AG, Kaiseraugst, Switzerland) for 20 weeks according to their assignment. Kidneys and livers were harvested 20 weeks after surgery and the mice were sacrificed. The isolated liver and kidney tissues were kept in 1.5ml Safe-Lock tubes and stored at -80°C.

3.2 RNA extraction

RNA extraction from kidney and liver samples was accomplished by using TRIzol® reagent (Life Technologies, USA). 1ml of TRIzol was added to each sample and the tissues were minced and homogenized by using 1.5ml pestles and a mixer (Polytron). To permit complete dissociation of the nucleoprotein complex the samples were incubated for 2-3min at room temperature. Subsequently, 0.2ml of chloroform was added and the samples were centrifuged (12‘000g, 15min, 4°C) to separate RNA from DNA and other cellular proteins. After centrifugation, RNA remained in an upper aqueous phase whereas DNA and proteins were present in the interphase and a lower phenol phase. The upper phase with the RNA was removed and transferred to new RNase-free tubes. Thereafter, 500µl of 100% isopropanol was added to precipitate the RNA within the aqueous phase. After a further centrifugation (12’000g, 15min, 4°C), an RNA pellet was formed at the bottom of the tube. The supernatant was removed and the pellet was washed twice with 80µl of 75% ethanol. After discharging the ethanol, the RNA pellets were left to air dry at room temperature for 5-10min. Depending on the pellet’s size 20-100µl of RNase-free water

were used to resuspend the pellet. The RNA concentrations were determined by spectrophotometry using Nanodrop (Witec AG, Switzerland). The sample purity was assessed by the $A_{260/280}$ ratio, which ideally ranged between 1.8 and 2.0.

3.3 cDNA synthesis

The High Capacity cDNA Reverse Transcription Kit (Life Technologies, USA) was used to synthesize single-stranded cDNA out of mRNA. 5µg RNA of kidney samples and 2µg RNA of liver samples were used for reverse transcription. The standard reaction mix and total reaction volume for 1µg RNA were adapted respectively and the RNA was mixed up with an appropriate amount of RNase-free water, half of the final reaction volume. The samples were incubated according to the scheme given by the manufacturer.

Table 1: Reverse transcription mixtures

	Standard	Kidney	Liver
RNA (µg)	1	5	2
RNA + ddH_2O (µl)	10	40	20
Reverse Transcription Buffer (µl)	2.0	8.0	4.0
dNTPs (µl)	0.8	3.2	1.6
Random Primers (µl)	2.0	8.0	4.0
Reverse Transcriptase (µl)	1.0	4.0	2.0
RNase Inhibitor (µl)	1.0	4.0	2.0
ddH_2O (µl)	3.2	12.8	6.4
Total reaction volume (µl)	20	80	40

3.4 Relative quantification of gene expression

Aliquots of synthesized cDNA were used as template for real-time PCR. Gene expression was analyzed using real-time PCR (TaqMan). For each target gene a master mixture containing primer and probe was prepared. Master mixture and sample aliquots were pipetted on a 384-well reaction plate. The reaction volume per well was 10µl, containing

2µl of the sample and 8µl master mixture. The samples were arranged in triplicates (3 wells per gene per sample). β-actin was used as housekeeping gene (reference gene) for both tissue types (kidney and liver). The $2^{-\Delta\Delta Ct}$ method [23, 24] was applied to analyze the changes in gene expression. The Ct (cycle threshold) values provided from real-time PCR were imported into a Microsoft Excel sheet. Means and standard deviations of Ct values were determined for target and reference gene in each sample. Using the $2^{-\Delta\Delta Ct}$ method, the data are presented as the fold change in gene expression normalized to β-actin and relative to the reference sample from the control group (sham chow). The fold change in the target gene normalized to β-actin and relative to the control, was assessed for each sample.

3.5 Immunohistochemical staining of Oatp1a1 in the liver

To evaluate the protein expression of Oatp1a1 in formalin-fixed paraffin-embedded liver sections the microwave based antigen retrieval technique was used. First, the sections were deparaffinized in two 5min changes of xylene, rehydrated through a graded series of ethanol (100%, 95% and 80%, 5min each) and rinsed in distilled water. After immersing in a staining dish containing 10 mM citrate buffer (pH 6.0), the sections were placed in the microwave for 3 cycles of 1 – 5min (each cycle until boiling point). Thereafter, the slides were left at room temperature to cool down for 20min and washed in distilled water. Subsequently, the slides were placed in 3%-H_2O_2 at room temperature for 20min, washed 3x 5min with PBS (phosphate buffered saline), immersed in 5% milk/PBS at room temperature for 30min and rinsed briefly in distilled water. The sections were incubated with the first rabbit anti-Oatp1a1 antibody (8) overnight at 4°C. Sections were again washed with PBS (3x 5min), HRP (Horseradish peroxidase)-polymer anti-rabbit antibody was added, slides were incubated at room temperature for 30 min and washed with PBS (3x 5min). HRP was detected using DAB (3,3'-Diaminobenzidine).

3.6 In silico analysis of Slco1a1 (Oatp1a1) promotor region

In silico analysis of the Slco1a1 promotor region was performed to identify putative FXR response elements (IR1, 5' AGTTCAnTGAACT 3'). 2000 base pairs upstream from the transcription start (NCBI Reference Sequence: NC_000072.6) were analyzed by visual inspection, using MatInspector (Genomatix software) and Nubiscan (University Basel).

3.7 Statistical analyses

Changes in gene expression were calculated in Microsoft Excel. The statistical analysis was performed with the GraphPad Prism software. A one-way ANOVA followed by a Bonferroni's test was used for comparison of the four groups. P-values < 0.05 were considered as significant.

3.8 List of materials and chemicals

1.5 ml Pestle (VWR International, USA)
1.5 ml Safe-Lock Tubes (Eppendorf AG, Germany)
2-Propanol ≥ 99.5% (Sigma-Aldrich, USA)
50 ml Polypropylene Conical Tube (BD Biosciences, USA)
Centrifuge 5417 R (Vaudaux-Eppendorf, Switzerland)
Centrifuge 5810 R (Vaudaux-Eppendorf, Switzerland)
Chloroform 99% (Sigma-Aldrich, USA)
Ethanol for molecular biology ≥99.8% (Merck KGaA, Germany)
GraphPad Prism software (GraphPad, USA)
High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Life Technologies, USA) containing:
10X RT Buffer
10X RT Random Primers
25X dNTP Mix (100 mM)
MultiScribe® Reverse Transcriptase (50 U/µL)
MicroAmp Optical 384-Well reaction plate (Applied Biosystems, Life Technologies, USA)
MicroAmp Optical Adhesive Film (Applied Biosystems, Life Technologies, USA)
Multipette plus (Vaudaux-Eppendorf, Switzerland)
NanoDrop ND-1000 Spectrophotometer (Witec AG, Switzerland)
Nuclease-free water (Ambion, Life Technologies, USA)
PicoFuge (Stratagene Cloning Systems, Agilent Technologies, USA)
PT 1200 C Mixer (Polytron, Kinematica AG, Switzerland)
RNAse free 1.5 ml Microfuge Tubes (Ambion, Life Technologies, USA)
TaqMan Fast Universal PCR Master Mix (Applied Biosystems, Life Technologies, USA)
Thermomixer comfort (Vaudaux-Eppendorf AG, Switzerland)
TRIzol Reagent (Ambion, Life Technologies, USA)
ViiA 7 Real-Time PCR System (Applied Biosystems, Life Technologies, USA)
Vortex-Genie 2 (Scientific Industries, USA)
Xplorer 5-100 ul (Vaudaux-Eppendorf, Switzerland**)**

4 Results

4.1 Effect of uninephrectomy and high fat diet on body weight

Mice fed with the high-fat diet had a markedly increased body weight compared to those with the normal chow diet. There was no significant difference in body weight between the UNX-mice and their counterparts with the sham surgery in both diet groups (Table 2).

Table 2: Body weight in the different groups after 20 weeks of diet

	Sham chow	Sham HFD	UNX chow	UNX HFD
Body weight in g (mean ± SD)	19.5 ± 0.6	28.8 ± 4.3	21.0 ± 0.6	29.2 ± 2.9

$p < 0.05$: sham-chow vs. sham-HFD, UNX-chow vs. UNX-HFD

$p > 0.05$: sham-chow vs. UNX-chow, sham-HFD vs. UNX-HFD

Abbreviations: SD, standard deviation; HFD, high fat diet; UNX, uninephrectomy

4.2 Gene expression in mouse kidney from different treatment groups

Membrane transporter genes along with FXR regulated genes in the mouse kidney were examined using semi-quantitative PCR analysis to determine the effect of diet-induced obesity and uninephrectomy on their expression and regulation. The genes selected for investigation in this study are listed in *Table 3*. *Figures 1* and *2* illustrate the PCR results. The expression of each gene is plotted as the fold change normalized to β-actin and relative to the control group (Sham-chow). The four groups were compared as follows: Sham-chow versus Sham-HFD, UNX-chow versus UNX-HFD, Sham-chow versus UNX-chow and Sham-HFD versus UNX-HFD.

Among the investigated Oatps in the kidney no significant changes in gene expression were observed with the exception of Oatp1a1 and Oatp1b2 (Figure 1). Mice fed with a high-fat diet showed a marked decrease of Oatp1a1 and Oatp1b2 in the kidney compared to the chow diet. Whereas Oatp1b2 expression was significantly lower in both Sham-HFD

and UNX-HFD mice, the shift in Oatp1a1 expression in the Sham-HFD group did not reach statistical significance compared with the sham-chow. There was no difference in the expression of Oat2 (organic anion transporter 2, gene Slc22a7) between the groups. Oat2 may not be affected by either HFD or UNX in the kidney (Figure 1). The expression level of FXR in the sham-HFD and UNX-chow groups seemed to be decreased, although the changes did not reach statistical significance. Shp (small heterodimer partner), which is regulated by FXR activation, was increased in the Sham-HFD and UNX-HFD mice; yet, its deviation in the UNX-HFD group was not statistically significant (Figure 2). Taken together, the impact of high-fat diet on renal Oatp gene expression was stronger than that of uninephrectomy. Moreover, an enhancing effect of UNX on changes in gene expression of transporters in the kidney could not be observed.

Table 3: List of tested genes

Gene symbol	Protein
Nr1h4	Fxr
Nr0b2	Shp
Slco1a1	Oatp1a1
Slco1a4	Oatp1a4
Slco1b2	Oatp1b2
Slco2b1	Oatp2b1
Slc22a7	Oat2

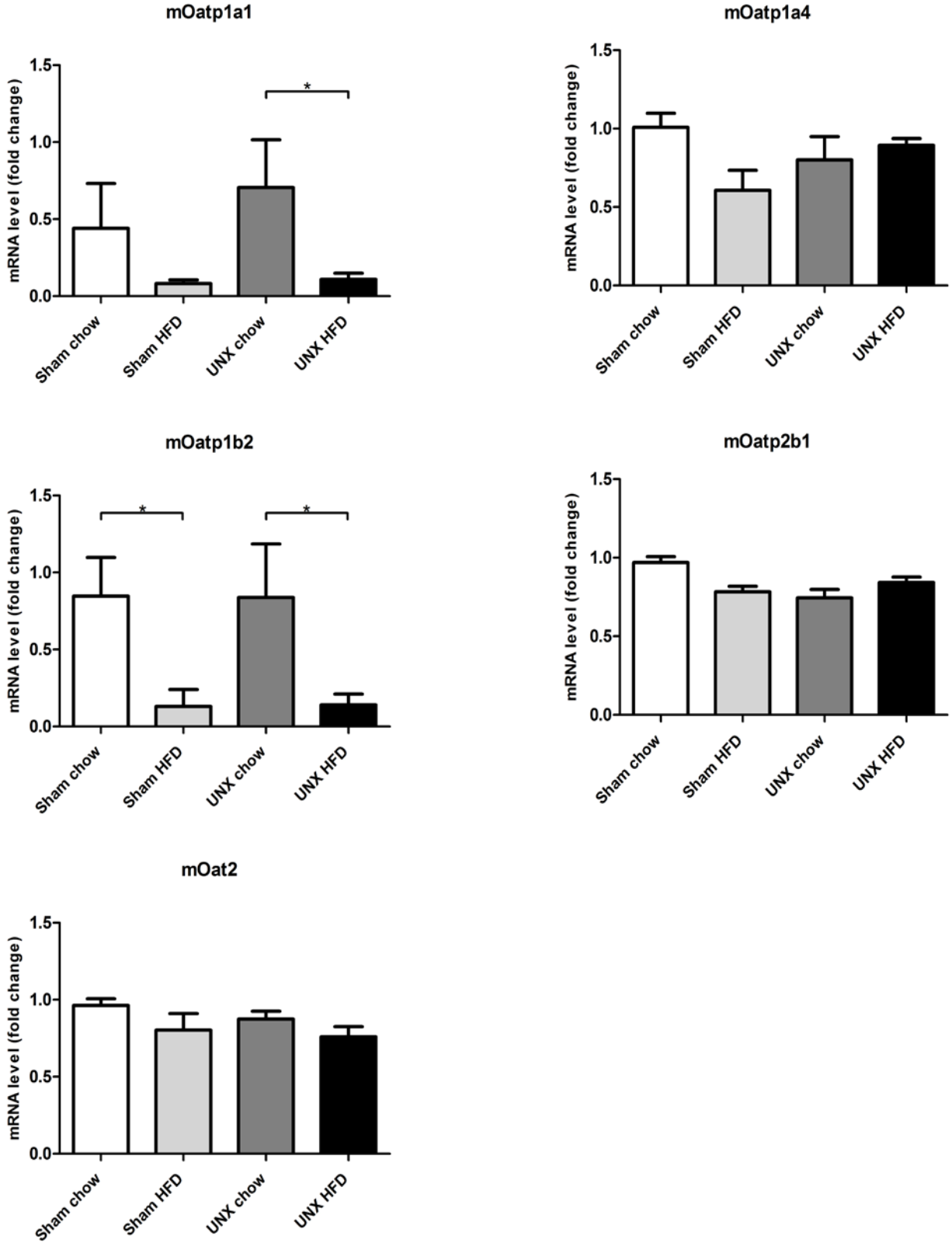

Fig. 1: Expression of Oatps and Oat2 in mouse kidney from different treatment groups. Data are shown as mean ± SEM, *p < 0.05

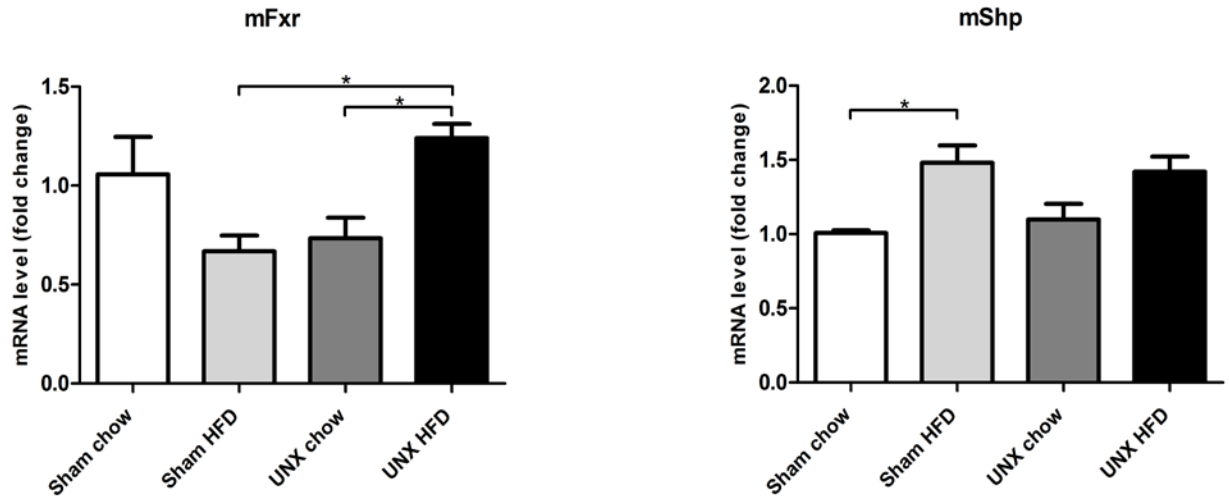

Fig. 2: mRNA expression level of FXR and Shp in the kidney from different treatment groups. Data are shown as mean ± SEM, *p < 0.05.

4.3 Gene expression in the liver

Since UNX could also affect bile acid transporters in the liver [25], expression analysis for the same genes as tested in the kidney (Table 3) was performed in liver tissues of the different treatment groups. Whereas Shp seemed to be increased in the Sham-HFD and UNX-HFD groups, the expression of Fxr revealed no relevant changes (Figure 3). Similar to the results in the kidney, Oatp1a1 expression in the liver was lower in the high-fat diet groups. In contrast to the findings in the kidney, the expression of Oatp1b2 was not altered in the liver. Oatp1a4 and Oatp2b1 showed no differences in expression level between the four groups (Figure 4). Furthermore, hepatic expression of Oat2 was decreased in mice fed with a high-fat diet. Similar to the observation in the kidney, the impact of a high-fat diet on hepatic gene expression was stronger than that of uninephrectomy. No additional effect of UNX on the changes in gene expression of transporters in the liver was observed.

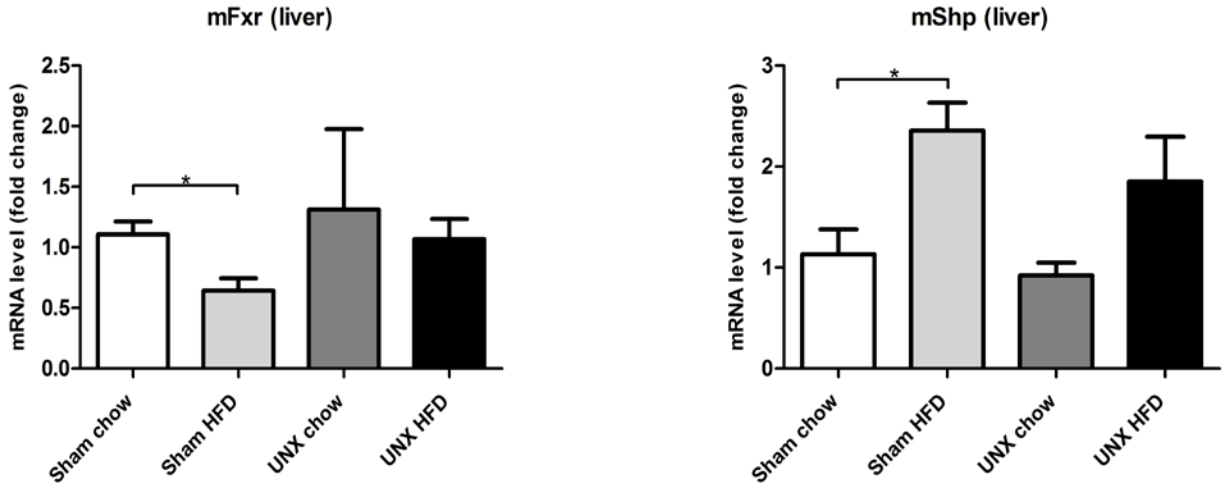

Fig. 3: mRNA expression of Fxr and Shp in mouse liver. Data are shown in mean ± SEM, *p < 0.05.

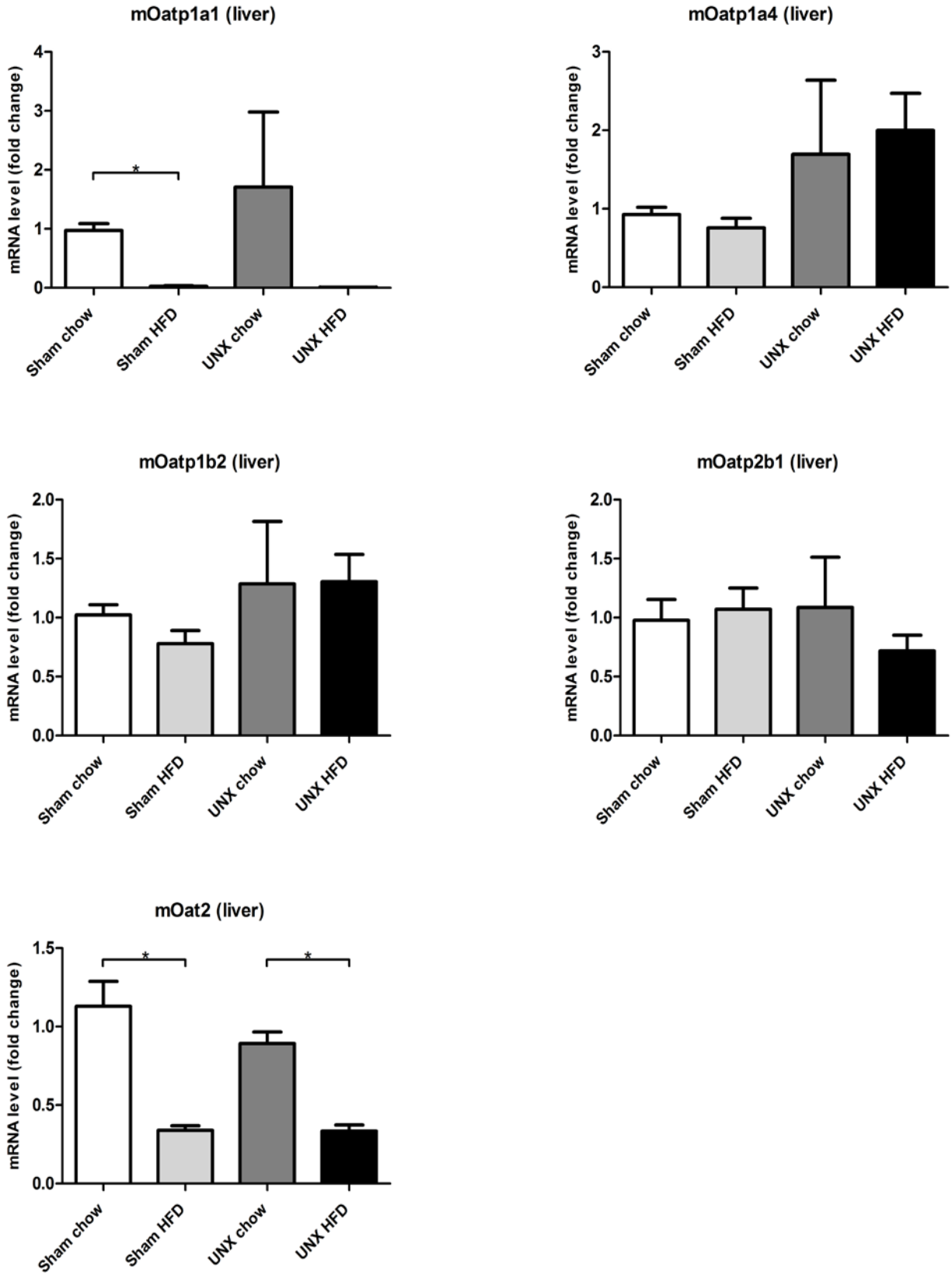

Fig. 4: Expression of transporter genes in mouse liver. Data shown as mean ± SEM, *$p < 0.05$.

4.4 Immunostaining of Oatp1a1 in the liver

To evaluate the protein expression of Oatp1a1, immunohistochemistry staining was performed in liver tissues comparing the sham-chow and sham-HFD groups. As shown in Figure 6, the expression of Oatp1a1 was lower in the sham-operated mice fed with a high-fat diet. Further, hepatic steatosis was observed in the Sham-HFD group.

Fig. 5: Immunohistochemical staining of liver samples with antibodies against Oatp1a1. The brown color indicates positive staining. *A)* Mice from the sham-chow/control group showed an unremarkable hepatic tissue and normal expression of Oatp1a1. *B)* Mice from the sham-HFD group with decreased expression of Oatp1a1 and hepatic steatosis as secondary finding.

4.5 In silico analysis of Oatp1a1 (Slco1a1) promotor region

Since Oatp1a1 has been reported as a FXR target gene [8], in silico analysis of the Oatp1a1 promotor region was performed to identify putative FXR response elements arranged as inverted repeats (IR1, 5' AGTTCAnTGAACT 3'). A region spanning 2000 base pairs upstream of the transcription start (NCBI Reference Sequence: NC_000072.6) was analyzed. Inverted repeats (IR1, 5' AGTTCAnTGAACT 3') could not be found in this fragment. However, several PPARα binding sites were found. As shown by Cheng et al. [26], Oatp1a1 expression can be repressed by activation of different transcription factors including aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), nuclear factor erythroid 2-related factor 2 (Nrf2), and PPARα (peroxisome proliferator-activated receptor). Considering the fact that the high-fat diet used in this study contained a high load of free fatty acids (coconut oil, a potent PPARα ligand), PPARα seemed to be the most probable among the nuclear receptors that were evaluated (not listed here). Typical response elements for PPARα are direct repeats, DR1 and DR2 sequences [27]. Table 4 shows the putative PPARα binding sequences reported by the program Nubiscan. The sequence 5' TGACCTaTGATCT 3' (AGATCAtAGGTCA on the negative strand) showed the best matching core elements and is depicted in Figure 6. Cheng et al. [28] mentioned this sequence as a putative PPARα response element in Oatp1a1 promotor.

Repeat	Position (strand)	p-value	Sequence (5’ to 3’)
DR1	1879 (-)	0.0019112	AGATCAtAGGTCA
IR0	1069 (+)	0.0034975	AGGGCATCACCT
DR8	1872 (-)	0.0045267	AGGTCAatctcattTGGACA
DR1	1145 (+)	0.0145049	AGGCCAgAGTTTA
DR7	487 (+)	0.0198807	AGGTCActtgttaAGTGGA
ER7	1874 (+)	0.0433879	TGATCTagcatgtAGTGGA
IR8	1853 (+)	0.0178256	TGTCCAaatgagatTGACCT
IR0	776 (+)	0.0538986	TGGTCATTTCCT
DR0	1139 (+)	0.0239962	AGCTTAAGGCCA

Table 4: List of putative PPARα response elements in the mouse Oatp1a1 promotor spanning 2000 base pairs proximal to the transcription start site. Sequences were identified using the program Nubiscan. Compared to the other sequences the DR1 (labeled) at the top had the best matching core elements in the sequence.

-390	TGCCAGATTGGCGCACTGCAGTGATCCTGTTCTTT-GGGTCTTCCCAGTGATGGGCTTACAGGCATGTGAA
-320	GAGAAGCCTAGCTCTTTTACTTGCCTGGTAA-TAATTAAAAGTCAGGGCCTCAA-GCTTAAAAAAAATCAAA
-250	CACTTTACCACTGAACCACCAC-CTCCCTATCTGTGCTGGACAGTTTTATATGAATTT-GACATTCACTAGA
-180	GTAATTTGGGAAGAGTGGCCTTCTGTTGA-GAAAAATGTCCAAATGAGATTGACCTATGATCTAG-CATGTA

-110	GTGCATTTTCTTTATTTATAATT- GACATGTGAAGGTCTAGCACATGG- TAGGTGGGTCTTGTTTGGTAGGT
-40	AGTCCTGGGTTGTATAAGAAGGTCAAGCAAGTCTT- GAGGA→(TS)

Fig. 6: Promotor region of Slco1a1 (Oatp1a1); 390 base pairs upstream from the transcription start (TS). Putative PPARα- binding site (labeled).

5 Discussion

It is well known that obesity is a physical condition with a range of short- and long-term health complications. A large body of literature indicates that obesity is a major risk factor for liver and kidney disease. It can be either the cause or an aggravating factor when other conditions such as hypertension and diabetes are established [4, 5, 29, 30]. To date, the pathophysiological mechanisms underlying obesity-related liver and kidney disease have not been fully clarified. There is increasing evidence that the endocrine and immunomodulatory properties of the adipose tissue contribute to the development of organ damage [5, 31, 32]. Moreover, in the recent years, several studies have revealed a great impact of obesity on the expression of renal and hepatic drug transporters including OATPs in obese and diabetic mouse models [7, 8, 33]. Among other cell-membrane-transporters, OATPs have attracted increasing interest for their role in drug disposition and metabolism. Due to their capacity to transport a wide range of xenobiotics including pharmaceuticals and toxins, the OATPs are considered to play essential roles in drug absorption, distribution and elimination. However, their physiological and pharmacological functions remain elusive and are yet subject of ongoing research.

The current study revealed a clear impact of obesity on renal and hepatic expression of Oatps in the high fat diet-induced obese mouse model. While the effect of the high-fat diet on Oatp expression was evident, uninephrectomy did not show a relevant impact on transporter expression in both kidney and liver. These findings suggest that the decrease in transporter expression is mainly caused by the high-fat diet.

Of interest, Oatp1a1 (Slco1a1) was decreased in both liver and kidney of obese mice after a 20-week-high-fat diet intervention. Moreover, the immunohistochemistry staining showed a markedly reduced expression of Oatp1a1 in the liver of obese mice (Figure 6). These results are in line with the previous results from our lab [8], which showed lower expression of Oatp1a1 in the kidney of obese mice. Other authors also described down-regulation of Oatp1a1 in obese, type 1 and type 2 diabetic mice [16, 33]. Oatp1a1 is highly expressed in the liver and kidney. Among other Oatps it is located at the basolateral membrane of hepatocytes and at the apical membrane of proximal tubules in the kidney. It mediates the transport of bile acids, steroid conjugates, hormones and numerous drugs [18, 34]. Digoxin, methotrexate, fexofenadine, and toxins such as phalloidin are known

substrates for Oatps [35-37]. However, pharmaceuticals, which are selectively transported through Oatp1a1, are still unknown. Knockout studies have shown that loss of Oatp1a1 changes the bile acid composition in the intestine and the profile of the intestinal microbiota. Furthermore, Oatp1a1-null mice were shown to have a markedly different bile acid profile in the feces, with a decrease in conjugated primary bile acids (taurine-, glycine-, and sulfate-conjugated bile acids) and an elevation of secondary bile acids [38]. Zhang et al. [39] also demonstrated that Oatp1a1-null mice are more sensitive to cholestatic liver injury. Moreover, due to its function as a drug transporter, a decrease of Oatp1a1 could alter the absorption, distribution and excretion of certain pharmaceuticals, leading to alterations of pharmacokinetics, efficacy and toxicity of substrate compounds. The fact that there are no human homologues of mouse Oatp1a1 with a high level of expression in hepatocytes [35, 36] may raise some question on the overall relevance of Oatp1a1 to hepatic drug metabolism in humans. However, Oatp1a1 has been shown to share a wide range of substrates with human OATP1B1 and 1B3 [18]. Thus, the functions of human OATP1B1 and 1B3 are also possibly preserved in mouse Oatp1a1. Hence, study in mice provides important clues for further investigation and tests in humans.

The regulation of Oatp1a1 has not been extensively studied, particularly with regard to nuclear receptor involvement. In silico analysis of the Oatp1a1 promotor region revealed no putative binding sites for FXR, but several PPARα response elements were found (Tab. 4, Fig.6). However, in silico analysis depends on the algorithm implemented and cannot claim completeness or correctness. While the best matching PPARα binding site (DR1, 5’ TGACCTaTGATCT 3’) found was detected by NUBISCAN (University Basel) and by visual inspection, it was surprisingly not detected by MatInspector Software. The fact that other authors [28] have described this sequence as a putative PPARα binding element supported our finding. However, only the minimal promotor spanning a 2 kb region was analyzed. FXR binding elements could be located outside of this selected fragment. It is well established that FXR typically binds to inverted repeats (IR1), but as demonstrated by Laffitte et al. [40], FXR can also modulate gene expression through direct repeats (DR4 and DR5 elements), which were not included in our analysis. Interestingly, FXR activation can induce PPARα expression [41]. Thus, it is possible that FXR modulates Oatp1a1 expression indirectly through PPARα activation. Interestingly, SHP, a FXR regulated transcription factor, seemed to be elevated in diet-induced obese mice (Figures 1, 3), although without significant change in the UNX mice. SHP is a member of

the “orphan” nuclear receptors and mainly acts as transcriptional repressor. SHP can be induced through several transcription factors (TFs) including FXR, HNF4α, PXR, and ERα and exerts its transcriptional activity through protein-protein interaction with those TFs. Fenofibrate, a PPARα agonist, has been shown to decrease Oatp1a1 expression in the mouse liver [26, 42]. It has also been shown to increase SHP gene expression in cultured liver cells and in normal as well as in diabetic mouse liver [43, 44]. Given the current findings from the gene expression analysis and the fact that the high-fat diet used had a high concentration of PPARα ligands (coconut oil), it is conceivable that the down-regulation of Oatp1a1 in this setting was at least partly mediated through PPARα.

Oatp1b2 expression in the kidney was significantly decreased in the high-fat diet groups, whereas the hepatic expression showed no difference between the four groups. Regarding the renal expression, the current findings are consistent with previous studies [8, 33]. However, Oatp1b2 is almost exclusively expressed in the liver and is considered the major liver-specific uptake transporter for drugs and other xenobiotics [45]. Oatp1b2 has two human orthologs (OATP1B1 and 1B3), and mediates the hepatic uptake of known OATP1B substrates such as pravastatin and rifampicin [46]. Meier zu Schwabedissen et al. [47] reported that most of the nonhepatic expression of Oatp1b2 appears due to splice variants of Oatp1b2. It is still not clear whether these splice variants are fully functional. The results of our study indicate that the expression of Oatp1b2 in the kidney might be affected by diet-induced obesity.

While the hepatic expression of Oat2 was decreased in mice fed with a high-fat diet, Oat2 expression in the kidney did not change after HFD. Other studies have demonstrated decreased Oat2 expression in kidney and liver of obese rodents [8, 33]. Oat2 belongs to the family of the organic anion transporters (gene symbol SLC22/Slc22) and has the highest expression levels in the liver with lower levels in the kidney. Typical substrates for Oat2 include salicylates, acetylsalicylate, prostaglandin E2, dicarboxylates, glutamate, and PAH (para-aminohippuric acid), as well as some antivirals. Oat2 also mediates the transport of propionate, a short-chain fatty acid, which is a natural ligand for receptors inducing leptin release. Thus, Oat2 may play a critical role in the regulation of leptin secretion [48]. Decreased expression of Oat2 could affect the metabolism of fatty acids and the pharmacokinetics of certain drugs. However, the relationship between altered metabolic state and Oat2 expression remains to be elucidated.

In conclusion, this study demonstrated that diet-induced obesity considerably alters renal and hepatic expression of xenobiotic transporters in mice, especially the organic anion transporting polypeptides Oatp1a1 and Oatp1b2. Moreover, in silico analysis suggests that the regulation of Oatp1a1 expression may involve several members of the nuclear receptor family of transcription factors. Further studies need to be conducted to elucidate the functional consequences of these changes in expression. Because some drug metabolizing enzymes and transporters are regulated similarly in mice and humans, the changes in the expression levels of homologous transporters observed in mice can be extrapolated to humans, with implications for the pharmacokinetics of substrate drugs in obese human individuals.

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8 Bestätigung der Eigenleistung

Dissertation

Ich erkläre ausdrücklich, dass es sich bei der von mir im Rahmen des Studiengangs

Humanmedizin

eingereichten schriftlichen Arbeit mit dem Titel

Effect of obesity on the expression of the organic anion transporting polypeptide Oatp1a1 in mouse liver

um eine von mir selbst und ohne unerlaubte Beihilfe sowie *in eigenen Worten* verfasste Dissertation handelt.

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Ich bestätige mit meiner Unterschrift die Richtigkeit dieser Angaben.

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9 Curriculum vitae

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